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Microbial Interference Between Indigenous Yeast and Lactobacilli in the Rodent Stomach

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Indigenous yeasts grow in layers in the mucus on the secreting epithelium of the stomachs of some strains of rats and mice raised under conventional conditions. Likewise, indigenous lactobacilli appear in layers on the nonsecreting epithelium of the stomachs of rats and mice. The two microbial layers can coexist in the same animals. When I gave such rodents penicillin solution in the place of drinking water, the lactobacilli disappeared, and the yeast from the secreting epithelium colonized the nonsecreting epithelium within 24 hr. The yeast remained in layers on the nonsecreting, as well as the secreting epithelium, as long as penicillin was administered. There is no inflammatory reaction or any sign that the yeast invaded below the keratin layer. When the penicillin treatment was discontinued, within 5 to 8 days the indigenous lactobacilli again colonized the nonsecreting epithelium. Concomitantly the yeast was displaced from the keratinized tissue and once more could be found only on the secreting epithelium. Only 2 days were required, however, for the bacteria to recolonize the keratin layer and displace the yeast when the mice were given indigenous lactobacilli in pure culture immediately after the penicillin treatment was discontinued. The lactobacilli must displace the yeast from the nonsecreting epithelium by interfering either with multiplication of the yeast on the tissue or with attachment of the yeast cells to the keratin layer. This interference must proceed continuously during normal life since the yeast never populates the nonsecreting epithelium as long as the lactobacilli are present.

Lactic acid bacteria, in particular certain lactobacilli, are known to suppress the growth in vitro of other bacteria and some fungi (6, 15). Lactobacilli of the normal microflora may suppress the growth of certain other microorganisms in the mammalian gastrointestinal tract (5, 14). Such growth competition is believed to be an important factor regulating the numbers and types of microorganisms on the surfaces of animal bodies (6).

This hypothesis was tested in experiments described in this report. Advantage was taken of the epithelial localization of lactobacilli and yeast in the rodent stomach (1, 7, 9). Yeasts of the genus *Torulopsis* grow in layers in the mucin on the surface and deep in the gland pits of the secreting epithelium of the stomachs of mice (7) and rats (1) from many conventionally housed colonies. Likewise, lactobacilli grow in layers on the keratinized squamous epithelium of the nonsecreting portion of the stomachs of rats (1) and mice (9) from both specific pathogen-free and conventionally housed colonies. The two microbial layers can coexist in the same animals and appear to be mutually exclusive (7). My data show that the yeast will colonize the nonsecreting epithelium when animals with such coexisting microbial layers are treated with penicillin. Additional evidence suggests that the failure of the yeast to colonize the nonsecreting epithelium under normal conditions is due to interference from the population of lactobacilli on that area of the stomach mucosa.

MATERIALS AND METHODS

Animals. Conventionally housed mice of the Swiss-Webster (SW) strain were obtained from the randomly bred colony of Thomas Euer's Farm, Austin, Texas. Specific pathogen-free (SPF) mice were obtained from the randomly bred Ha/ICR strain colony of the E. R. Schmidt Co., Madison, Wis. Conventionally raised rats were obtained from the randomly bred colony of an unknown albino strain (the so-called Houston-Cheek rat) of the Cheek-Jones Co., Houston, Texas. The conventionally raised animals were housed in plastic or metal cages. SPF animals were housed in plastic cages with paper tops (Carworth, New City, N.Y.). All animals were given LabBlox (Allied Mills, Chicago, Ill.) and acid-water (10). Only 6- to 8-week-old males were used.

Microbial cultures. A pure culture of yeast from the stomachs of SW mice was isolated on Sabouraud Dex-

trose Agar (Difco) incubated at 37 C. This yeast reproduced asexually by budding and grew poorly or not at all at room temperature. Thus, it was recognized provisionally as a strain of *Torulopsis pintolopesii* as described by Van Uden (13). It was maintained in Microassay Broth (Difco).

Three pure cultures of lactobacilli from the stomachs of Ha/ICR mice were isolated on 10A medium (10) and incubated in a candle jar at 37 C. These cultures were differentiated by colony form only; the colony forms approximated those described for lactobacilli isolated by Schaedler and Dubos (10) from the stomachs of NCS mice. These bacterial cultures were also maintained in Microassay Broth.

Quantitative culture techniques. Viable counts of yeast and lactobacilli were made by previously described procedures (7, 10, 11) from suspensions of gastric tissue. After the rodents were killed with chloroform, their stomachs were removed and a small portion of both the secreting and nonsecreting tissue was taken for histological examination. The remainder of the secreting mucosa of the stomachs was separated from the nonsecreting mucosa along the cardiac antrum. The antrum was dissected away and discarded to provide a clean separation between the two types of mucosal tissue. The two pieces of tissue were then separately weighed and ground in charcoal water in a tissue grinder. The resulting suspensions were used directly for viable counts.

Histological techniques. Portions of rodent stomachs with their contents intact were frozen at −22 C. Sections of the frozen tissues were made on a microtome-cryostat (International Equipment Co., Needham Heights, Mass.). The sections were fixed in absolute methanol and stained by the Gram method modified for tissues (9).

RESULTS

Colonization of the nonsecreting epithelium by indigenous yeasts in conventionally raised mice and rats given penicillin solution in the place of drinking water. SW mice and Houston-Cheek rats were given aqueous penicillin solution (0.3 g/liter) in place of drinking water. Every day for 8 days after the penicillin treatment was started, individuals or small groups of animals of both species were killed. The secreting and nonsecreting portions of the stomach mucosa from each animal were then cultured and examined histologically for both yeasts and lactobacilli (Table 1).

Both yeast and lactobacilli could be cultured from the stomachs of conventionally raised rodents not treated with penicillin. Consistently more yeast was cultured from secreting than nonsecreting tissue, whereas more lactobacilli were cultured from nonsecreting tissue. Histological examination of the stomachs of numerous individuals confirmed that yeast layers existed only on the secreting epithelium, and lactobacillus layers existed only on the nonsecreting epithelium in these animals.

In contrast to the findings with the untreated rodents, the lactobacilli disappeared from the stomachs of both mice and rats given penicillin solution within 2 days after the drug treatment was started. Concomitantly the numbers of yeast increased on the nonsecreting side almost to the levels found on the secreting side. Histological examination of stomachs of such treated mice showed that the yeast had colonized the keratinized epithelium of the nonsecreting tissue (Fig. 1–2). The yeast was never observed in deep tissues; no inflammatory reaction was ever seen.

Time required for the yeast to colonize the nonsecreting epithelium. SW mice were given aqueous

TABLE 1. *Colonization of the nonsecreting epithelium by indigenous yeasts normally found only on the secreting epithelium in the stomachs of penicillin-treated conventional rodents*

Type of animals	No. of animals	Drug treatment[a]	Stomach tissue cultured	Microorganisms cultured from the tissues[b]		Occurrence of stomach layers[c]	
				Yeast	Lactobacilli	Yeast	Lactobacilli
Mice	20	None	Secreting	8 (7–8)	8 (6–8)	12/12	0/12
			Nonsecreting	6 (4–6)	9 (8–9)	0/12	12/12
	18	Penicillin	Secreting	8 (7–9)	NC	18/18	0/18
			Nonsecreting	8 (6–9)	NC	17/18	0/18
Rats	14	None	Secreting	8 (7–9)	8 (7–9)	4/4	0/4
			Nonsecreting	5 (4–6)	9 (8–9)	0/4	4/4
	19	Penicillin	Secreting	8 (7–9)	NC	7/8	0/8
			Nonsecreting	7 (6–8)	NC	7/8	0/8

[a] Aqueous penicillin solution (0.3 g/liter) was given in the place of drinking water for 1 to 8 days before cultures were made. The results were constant after only 1 day of penicillin treatment (see Table 2). Therefore, the data taken over the 8 days were pooled for this table.

[b] Results are given as the arithmetical mean and range (in parentheses) of logarithms of the numbers of microorganisms cultured per gram of fresh tissue; NC, no microorganisms cultured.

[c] Expressed as the ratio of animals with stomach layers to the total number examined histologically.

penicillin solution in place of drinking water. Small groups of these treated mice were then killed at intervals, and their stomachs cultured for yeast and lactobacilli (Table 2). Within 24 hr, all of the lactobacilli had disappeared and the yeast was well established on the nonsecreting as well as the secreting sides.

Colonization by yeast isolated from SW mice of the secreting epithelium of the stomachs of specific pathogen-free Ha/ICR mice. As previously reported (7), yeast layers do not occur in the stomachs of mice of all strains and colonies. In particular, SPF mice are free of the layer-forming yeast. Such is the case with SPF mice of the Ha/ICR strain. SPF mice are normally healthy animals with gastrointestinal microfloras that are qualitatively and quantitatively simpler than the microfloras commonly found in conventionally raised animals. Consequently, experiments on gastrointestinal microflora usually yield more reproducible results when conducted in SPF rather than conventionally raised mice (10). For this reason, the yeast-lactobacillus interference phenomenon has been explored further in SPF Ha/ICR mice deliberately colonized with yeast isolated from SW mice.

Table 3 shows the time required for the yeast to establish in the stomachs of Ha/ICR mice given food contaminated with a pure broth culture of the SW yeast. At 2 to 3 days after contamination the numbers of yeast cultured from these artificially colonized Ha/ICR mice were the same as the numbers obtained from the naturally colonized SW mice (Table 1). Histological examination confirmed that a yeast layer had established on the secreting epithelium of the

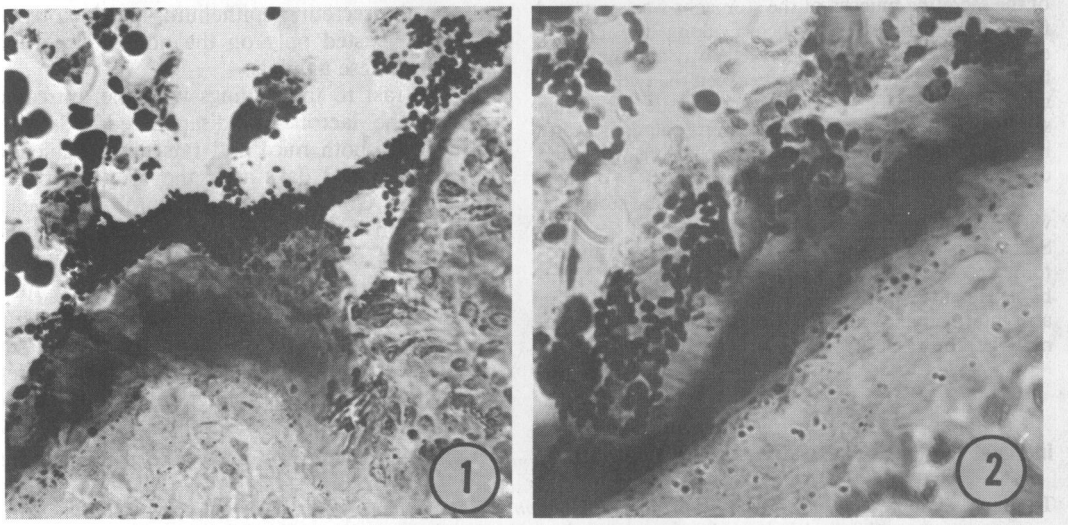

FIG. 1–2. *Gram-stained tissue sections showing yeast layers on the keratinized squamous epithelium of the nonglandular mucosa of the stomachs of SW mice given penicillin solution (0.3 grams/liter) in place of drinking water. Yeast layer at junction between nonglandular tissue (Fig. 1; left) and glandular tissue (right); × 750. Yeast layer on keratinized tissue (Fig. 2); × 1200.*

TABLE 2. *Culture results showing speed of colonization of the nonsecreting epithelium by indigenous yeast from the secreting mucosa in the stomachs of penicillin-treated SW mice*[a]

Microorganism cultured	Stomach tissue cultured	Hrs after penicillin treatment started[b]				
		0	6	24	72	120
Lactobacilli	Secreting	8 (6–8)	7 (6–8)	NC	NC	NC
	Nonsecreting	9 (8–9)	9 (8–9)	NC	NC	NC
Yeast	Secreting	8 (7–8)	8 (7–8)	8 (7–9)	8 (7–9)	8 (7–9)
	Nonsecreting	6 (4–6)	6 (4–6)	8 (4–9)	7 (6–9)	8 (7–9)

[a] Values are expressed as in Table 1; 15 animals per group.
[b] Aqueous penicillin solution (0.3 g/liter) was given in the place of drinking water.

stomachs of the SPF animals within 2 days after the contamination. Such Ha/ICR mice contaminated with yeast, as well as ones not contaminated with yeast, possessed a *Lactobacillus* layer on the nonsecreting epithelium of their stomachs.

Colonization of the nonsecreting epithelium of the stomachs of Ha/ICR mice first contaminated with yeast then given penicillin solution in the place of drinking water. Ha/ICR mice were contaminated with the SW yeast as explained above. At 2 or 3 days after the yeast contamination, at which time layers of yeast existed on the secreting epithelium of their stomachs, the animals were given aqueous penicillin solution (0.3 g/liter) in the place of drinking water. Thereafter, every day for 8 days, some of the mice were killed, and their stomach mucosa cultured and examined histologically for yeast and lactobacilli (Table 4).

As in the SW mice that were normally colonized by the yeast, penicillin treatment removed the lactobacilli from the stomachs of Ha/ICR strain animals that had been artificially colonized by the yeast. Also, as in the SW mice, within 24 to 48 hr after beginning the penicillin treatment, the yeast had colonized the nonsecreting epithelium of the stomachs of the SPF animals.

Recolonization by lactobacilli of the nonsecreting gastric epithelium colonized by yeast during penicillin treatment of yeast-contaminated Ha/ICR mice. Ha/ICR mice were contaminated with SW yeast. After 3 to 5 days, when the yeast had thoroughly colonized the gastric secreting epithelium, the animals were divided into four groups. One group was given no further treatment. The second group was given aqueous penicillin solution (0.3 g/liter) in the place of drinking water, and this treatment was maintained throughout the experiment. A third group was given the penicillin solution to drink for only 5 days, at which time the drug treatment was discontinued and acid water was again given. The fourth group was also given penicillin solution for 5 days and, at the end of that period, given acid water to drink. In this case, however, at the end of the 5 days of penicillin treatment, the food and drinking water of the animals were heavily contaminated with pure cultures of 3 types of indigenous lactobacilli. Both before and at intervals after the lactobacillus contamination

TABLE 3. *Culture results showing the rate of colonization by yeast from SW mice of the secreting gastric epithelium of originally yeast-free Ha/ICR mice*

Microorganism cultured	Stomach tissue cultured	Days after yeast was given[a]				
		0	1	2	3	14
Lactobacilli	Secreting	7 (5–8)	7 (5–8)	7 (5–8)	7 (5–8)	7 (5–8)
	Nonsecreting	9 (8–9)	9 (8–9)	9 (8–9)	9 (8–9)	9 (8–9)
Yeast	Secreting	NC	<4	8 (6–8)	8 (6–8)	8 (7–8)
	Nonsecreting	NC	NC	4 (<4–5)	4 (4–6)	5 (4–6)

[a] Results are given as in Tables 1–2; 15 to 20 mice per group. On day 0, 10 to 20 ml of a pure 18-hr broth culture of SW yeast was poured over food pellets placed inside the cages.

TABLE 4. *Yeast colonization of the nonsecreting epithelium of the stomachs of penicillin-treated Ha/ICR mice previously given yeast isolated from SW mice*[a]

Drug treatment	Stomach tissue cultured	Microorganisms cultured from the tissues		Occurrence of stomach layers	
		Yeast	Lactobacilli	Yeast	Lactobacilli
None	Secreting	8 (7–8)	7 (5–8)	14/15	0/15
	Nonsecreting	5 (4–6)	9 (8–9)	0/15	15/15
Penicillin	Secreting	8 (7–9)	NC	21/21	0/21
	Nonsecreting	8 (6–9)	NC	20/21	0/21

[a] See Table 3 for the method of giving yeast to the mice. At least 3 to 4 days after yeast was given, aqueous penicillin (0.3 g/liter) was given in place of drinking water to 50 mice, with the remaining 50 serving as a control. Cultures were made 1 to 8 days after the penicillin treatment was started. The results were constant after only 1 day of penicillin treatment. Therefore, the data taken over the 8 days were pooled for this table. Values of microorganisms cultured are expressed as in Tables 1–3. Occurrence of stomach layers is expressed as in Table 1.

of the animals of the fourth group, a number of animals from each group were killed. Their stomachs were then cultured and examined histologically for yeast and lactobacilli (Table 5).

In summary, within 6 days after the penicillin treatment was discontinued, indigenous lactobacilli reestablished on the nonsecreting gastric epithelium. Concomitant with this recolonization by bacteria the number of yeasts on the nonsecreting tissue dropped to the levels observed before penicillin treatment. Consequently, the lactobacilli are able to displace the yeast from their locations on the nonsecreting tissue. The latter conclusion is reinforced by the findings with the animals given lactobacilli in pure culture after the penicillin treatment was stopped. In these animals the lactobacilli colonized the nonsecreting tissue and displaced the yeast within 2 days. Clearly, the lactobacilli are able to interfere with the growth of the yeast or with its attachment to the keratinized tissue.

DISCUSSION

Antibacterial drugs given orally rapidly and markedly alter the gastrointestinal microflora of specific pathogen-free mice (2, 8). Moreover, a microflora characteristic for each antibacterial drug will establish in the alimentary canals of such mice if the treatment is continued for prolonged periods (2). Each of these characteristic floras differs in both numbers and types of microorganisms from the flora found in untreated animals. However, soon after the drug treatment is discontinued the microbial elements of the normal flora reestablish (2, 8). Thus, the microorganisms in the drug-induced flora are able to colonize the stomach and gut only as long as the antibiotic suppresses the normal flora.

In like manner, certain microbial pathogens may also be permitted access to animal tissues when antibacterial drugs suppress the normal microflora. Pertinent to the present report is the finding that *Candida albicans* infections of mice are potentiated by treatment with antibacterial drugs (12). In addition *C. albicans* more readily colonizes the alimentary canals of mice given such antimicrobials (4). These findings probably should not be interpreted solely in terms of microbial interference at this time. However, the finding that lactobacilli can prevent yeast colonization of a body surface supports a hypothesis that *C. albicans* is inhibited from colonizing the guts and tissues of mice at least in part by elements of the normal microbial flora.

Although it seems clear that microbial competition operates in the mammalian gastrointestinal tract, it is equally clear that the mechanisms of such competition are not known. The model in which indigenous lactobacilli compete with indigenous yeast on a gastric epithelial surface may be useful in studying the mechanisms in vivo. In this regard, it is important to stress that the competition between the lactobacilli and yeast takes place on a particular epithelial surface: the keratinized squamous epithelium of the stomach. In mice populated by both yeast and lactobacilli, enormous numbers of the bacteria and some of the yeast as well can be cultured from this nonsecreting epithelium. However, the microbial layer on that tissue appears to consist only of bacteria

TABLE 5. *Culture results showing displacement of yeast from the nonsecreting epithelium by lactobacilli given to Ha/ICR mice previously given yeast from SW mice and then treated with penicillin*

Penicillin treatment[a]	Lactobacillus treatment[b]	Microorganism cultured	Microorganisms cultured from nonsecreting epithelium[c]			
			Day before lactobacillus treatment	Days after lactobacillus treatment		
				2	4	6
None	None	Lactobacilli	9 (8–9)	9 (8–9)	9 (8–9)	9 (8–9)
		Yeast	5 (4–6)	5 (4–6)	5 (4–6)	5 (4–6)
Continuous	None	Lactobacilli	NC	NC	NC	NC
		Yeast	8 (6–9)	8 (6–9)	8 (6–9)	8 (6–9)
5 days only	None	Lactobacilli	NC	NC	NC	9 (7–9)
		Yeast	8 (6–9)	8 (6–9)	8 (7–9)	5 (4–7)
5 days only	Yes	Lactobacilli	NC	9 (7–10)	9 (8–10)	9 (8–10)
		Yeast	8 (6–9)	6 (5–9)	6 (5–8)	6 (4–6)

[a] Aqueous penicillin solution (0.3 g/liter) was given in the place of drinking water for the time indicated.

[b] The day penicillin treatment was discontinued, 10 to 20 ml of pure 18-hr broth cultures of 3 different colony forming types of indigenous lactobacilli were poured on the food and into the drinking water.

[c] Data are given as in Tables 2–4. NC, no microorganisms cultured; 15 to 20 animals per group.

in histological sections. In contrast, the microbial layer on the secreting tissue is made up of yeast, although lactobacilli in significant numbers, in addition to the yeast, can be cultured from this secreting tissue. Thus, the yeast normally can colonize and form a layer on the secreting epithelium in spite of the lactobacilli that can be cultured from that area, but cannot form a layer on the nonsecreting tissue when the lactobacillus layer is present. It would seem, therefore, that the bacteria must be attached to the tissue in order to prevent yeast colonization. Since the lactobacilli only attach to the keratinized layer, the environment on the keratin must be a component of the competitive interaction.

It is now believed that at least two of the bacterial types that are considered elements of the autochthonous microflora of the gastrointestinal tracts of mice (3) are localized on epithelial surfaces. Lactobacilli form layers on the nonsecreting epithelium of the stomach (9). Fusiform rods form layers on the epithelium of the cecum and colon (9). The lactobacilli can prevent yeast colonization of the nonsecreting gastric epithelium. The fusiform-shaped bacteria may displace coliforms and enterococci from the mucin on the epithelium during their colonization of the large bowels of infant mice (9). Although the latter observation remains to be validated, it now seems likely that autochthonous bacteria can interfere with colonization of certain tissue surfaces by nonautochthonous microorganisms. Microbial competition on epithelial surfaces of tissues may be an important component in the regulation of the numbers, types, and localization of microorganisms in the normal microbial flora.

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